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Mitochondrial Control of Cell Death Induced by HIV-1-Encoded Proteins

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ABSTRACT

In most examples of physiological or pathological cell death, mitochondrial membrane permeabilization (MMP) constitutes an early critical event of the lethal process. Signs of MMP that precede nuclear apoptosis include the translocation of cytochrome *c* and apoptosis-inducing factor (AIF) from mitochondria to an extra-mitochondrial localization, as well as the dissipation of the mitochondrial transmembrane potential. MMP also occurs in HIV-1-induced apoptosis. Different HIV-1 encoded proteins (Env, Vpr, Tat, PR) can directly or indirectly trigger MMP, thereby causing cell death. The gp120/gp41 Env complex constitutes an example for an indirect MMP inducer. Env expressed on the plasma membrane of HIV-1 infected (or Env-transfected) cells mediates cell fusion with CD4/CXCR4-expressing uninfected cells. After a cell type-dependent latency period, syncytia then undergo MMP and apoptosis. Vpr exemplifies a direct MMP inducer. Vpr binds to the adenine nucleotide translocator (ANT), a mitochondrial inner membrane protein which also interacts with apoptosis-regulatory proteins from the Bcl-2/Bax family. Binding of Vpr to ANT favors formation of a non-specific pore leading to MMP. The structural motifs of the Vpr

protein involved in MMP are conserved among most pathogenic HIV-1 isolates and determine the cytotoxic effect of Vpr. These data suggest the possibility that viruses employ multiple strategies to regulate host cell apoptosis by targeting mitochondria.

Key Words: HIV • AIF, Bcl-2 • Caspases • Apoptosis • Cytochrome *c* • gp120 • Vpr

Abbreviations: [e] AIF, apoptosis inducing factor • ANT, adenine nucleotide translocator • COX, cytochrome *c* oxidase • Cyt.c, cytochrome *c* • Δ • Ψ • $_m$,mitochondrial transmembrane potential • Env, envelope glycoprotein complex • HIV, human immunodeficiency virus • MMP, mitochondrial membrane permeabilization • PBL, peripheral blood lymphocytes • Z-VAD.fmk, N-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone.

INTRODUCTION MITOCHONDRIAL CONTROL OF APOPTOSIS

Apoptosis research has recently experienced a change from a paradigm in which the nucleus determined the apoptotic process [1] to a paradigm in which caspases [2] and, more recently, mitochondria constitute the center of death control. [3-5] Today, it is almost generally accepted that the mitochondrial membrane constitutes (one of) the battle ground(s) on which opposing molecules determine whether cell death ensues. [5-10] Several observations support the hypothesis that mitochondria control cell death.

- First, mitochondrial membrane permeabilization (MMP [e]) mostly precedes the signs of advanced apoptosis or necrosis, irrespective of the cell type or the death-inducing stimulus. Mitochondria are organelles with two well-defined compartments, the matrix surrounded by the inner membrane (IM) and the intermembrane space surrounded by the outer membrane (OM). Apoptotic OM permeabilization involves the release of proteins which are normally confined to the intermembrane space of these organelles. [11-17] As compared to the OM, apoptotic IM permeabilization is selective, not resulting in a massive leakage of matrix proteins. IM permeabilization may occur in a step-wise and reversible fashion, with increasing permeability to solutes up to ~1500 D, culminating in the dissipation of the proton gradient responsible for the transmembrane potential ($\Delta\Psi_m$). [18-20]
- Second, MMP has a better predictive value for cell death than other parameters including caspase activation. As an example, crosslinking of the CXCR4 surface receptor or transfection-enforced overexpression of the tumor suppressor gene PML induce apoptosis with MMP but without detectable caspase activation. [21,22] Even if inhibition of caspases blocks some of the morphological and biochemical manifestations of apoptosis, it fails to prevent MMP and cell death induced via a variety of receptors (e.g., glucocorticoid receptor, CD2, CD4, CD45, CD47, CD99, MHC class II), anti-oncogenes (e.g., *Bax, Bak*), chemotherapeutic agents, irradiation, and

pro-apoptotic second messengers (e.g., reactive oxygen species, nitric oxide, ceramide, ganglioside GD3) (reviewed in refs. [8, 23]). Caspase activation can even be required for the transmission of mitogenic signals [24,25] and thus may be uncoupled from cell death.

- Third, an increasing number of pro-apoptotic effectors directly induce MMP when added to purified mitochondria *in vitro*. This applies to pro-apoptotic members of the Bcl-2 family such as Bax, [26] pro-apoptotic signal transducing molecules such as Ca^{2+}, ceramide, ganglioside GD3, fatty acids and their oxidation products, reactive oxygen species, nitric oxide, and cytotoxic agents including arsenite, betulinic acid, CD437, lonidamine, and photosensitizing porphyrin derivatives (reviewed in refs. [5, 23]).
- Fourth, anti-apoptotic members of the Bcl-2 family interact with mitochondrial membranes or membrane proteins and inhibit cell death by virtue of their capacity to prevent MMP. [11,27] Similarly, inhibition of mitochondrial membrane permeabilization by specific pharmacological interventions (that is by agents acting on mitochondrial proteins) prevents or retards cell death. This applies to N-methyl-4-valine-cyclosporin A (a ligand of the mitochondrial cyclophilin D), [28] bongkrekic acid (a ligand of the adenine nucleotide translocator), [29] and, in some experimental systems, oligomycin (an inhibitor of the F1 ATPase). [30]
- Fifth, cell-free systems have identified soluble intermembrane proteins (SIMPs) from mitochondria that are rate-limiting for the activation of catabolic hydrolases, mainly caspases and nucleases. The heme protein cytochrome *c*, for example, triggers the assembly of a caspase-9/caspase-3 activation complex, the apoptosome, in the cytosol. [31] Released mitochondrial hsp10 and hsp60 may also facilitate caspase activation. [32] Certain caspases can be found sequestered within mitochondria and, when liberated, may contribute to setting off caspase activation cascades. [14,15] Some SIMPs stimulate caspase-independent catabolic events. Once released from mitochondria, for instance, "apoptosis-inducing factor" (AIF), a flavoprotein oxidoreductase, is imported into nuclei where it stimulates large scale DNA fragmentation to ~50 kbp and peripheral chromatin condensation. [13,17] Additional catabolic enzymes released from mitochondria include arginase, sulfite oxidase, glycine cleavage system h protein, and soluble epoxide hydrolase. [33]

Altogether, these observations suggest a three-step model of apoptosis: a premitochondrial phase during which signal transduction cascades or damage pathways are activated (initiation phase); a mitochondrial phase, during which mitochondrial membranes are permeabilized (decision/effector phase); and a post-mitochondrial phase during which proteins released from mitochondria cause the activation of catabolic enzymes (degradation phase). This scheme, initially developed by our group, [3,4,34] has now been adopted by many of the leading scientists in the field. [7,9,10,35]

IMPACT OF HIV-1 INFECTION ON MITOCHONDRIA *IN VIVO* AND *IN CELLULA*

Peripheral blood lymphocytes (PBL) from HIV-1 infected patients are notoriously prone to spontaneously undergo apoptosis upon *in vitro* culture. [36] Apoptosis mainly affects cells in which the endogenous MMP inhibitor Bcl-2 has been down-regulated. [36] Interleukin-2 and interleukin-15, which up-regulate Bcl-2, inhibit spontaneous apoptosis of PBL. [37,38] Both $CD4^+$ and $CD8^+$ cells from the peripheral blood of HIV-1 infected individuals exhibit an increased susceptibility to lose the $\Delta\Psi_m$ *ex vivo*, before they manifest signs of nuclear apoptosis. [39,40] This latter result has been confirmed for peripheral blood lymphocytes from patients shortly after primary infection. [41] PBL from chronically HIV-1 infected donors also have an enhanced capacity to produce superoxide anion [39,40,42] in a reaction that is suppressed by the respiratory chain inhibitor rotenone, [40] suggesting that superoxide anion is generated in mitochondria. Moreover, in freshly isolated lymph node cells from HIV-1-infected donors, mitochondrial swelling has been detected by electron microscopy *ex vivo*. [43] These results suggest that cell death induced by HIV-1 is associated with MMP.

A further hint to a general mitochondrial effect of HIV-1 has been obtained by treating transgenic mice expressing replication-incompetent HIV-1 (NL4-3 Δ gag/pol) with 3'-azido-2',3'-deoxythymidine (AZT). [44] AZT, as well as analogous anti-retroviral nucleosides, negatively affect mitochondrial DNA replication and can provoke a persistent mitochondrial myopathy in AIDS patients, especially in infants. [45] In mice, transgenic HIV sensitizes to AZT-induced myocardial mitochondriopathy, [44] suggesting direct or indirect HIV-mediated effects on mitochondria. A similar result was obtained when AZT was administered to mice expressing the HIV-1 one-exon-encoded 72 amino acid Tat protein and full-length 86 amino acid Tat proteins. In Tat-expressing mice, AZT-treatment caused a greatly enhanced suppression of the mitochondrial isoform of superoxide dismutase (Mn-SOD or SOD-2), when compared to control mice. In addition, Tat exacerbated signs of AZT-induced (presumably mitochondrion-generated) oxidative stress. [46] Thus, HIV-1 and specifically Tat can affect mitochondrial metabolism *in vivo*, in the intact organism.

When T cells are infected with HIV-1 *in vitro*, they manifest mitochondrial swelling [47,48] and lose the expression of several nucleus-encoded mRNA species coding for mitochondrial proteins. [49,50] In addition, HIV-1 downregulates the cytochrome *b* mRNA from the mitochondrial genome. [50] Downregulation of mitochondrial DNA transcription is also a distinctive feature of apoptosis. [51] Cytopathogenic HIV-1 infected cells overproduce superoxide anion in mitochondria [52] and manifest a decline in the $\Delta\Psi_m$. [53] CD4-expressing HeLa cells co-cultured with a lymphoid cell lines chronically infected with a syncytium-inducing HIV-1 isolate exhibit the release of AIF and cytochrome *c* from mitochondria clearly before nuclear apoptosis occurs. [54] (Fig. 1). In U937 or Jurkat cells, overexpression of Bcl-2 or Bcl-X_L decreases HIV-1-induced apoptosis. [55,56] Bcl-2 overexpression has also been reported to suppress syncytial apoptosis in HIV-1 infected CD4 T cell lines. [57] These data suggest that HIV-1 affects mitochondrial function and causes a type of apoptosis which, at least in some cell types, depends on the (Bcl-2/Bcl-X_L inhibitable) MMP.

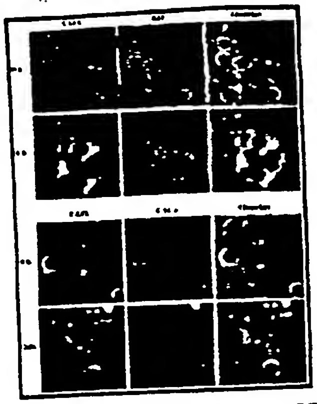

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FIGURE 1. Translocation of AIF translocation and cytochrome *c* in HIV-1 infected cells. CD4-expressing HeLa cells were either cultured alone (Co.) or co-cultured with chronically HIV-1-infected H9/IIIB cells at a 2:1 ratio. At the indicated time, cells were fixed and permeabilized to assess the subcellular localization of AIF (*upper panels*) or that of cytochrome *c* (*lower panels*), detected by fluoresceine isothiocyanate-labeled antibodies, in relationship to mitochondria (*both upper and lower panels*), detected with phycoerythrin-conjugated antibodies directed against cytochrome *c* oxidase, COX, a sessile inner membrane protein. Note the punctate staining pattern for AIF and cytochrome *c* in controls (overlapping with the staining pattern of COX) and the more diffuse pattern in HIV-1-induced syncytia. Note also the nuclear AIF staining pattern of syncytia.

EFFECT OF INDIVIDUAL HIV-1-ENCODED PROTEINS ON MITOCHONDRIA

HIV-1 infection induces apoptosis via a cornucopia of mechanisms, and there is considerable debate about which viral protein is the dominant inducer, either in HIV-1 infected cells or in non-infected "bystander" cells. Extracellular addition of several HIV-1 encoded proteins to cultured cells, as well as transient transfection of cells with the corresponding genes, induces apoptosis. This applies to Tat, Vpr, Nef, and Env. In contrast, cell lines stably transfected with either Vpr or Tat may become resistant to exogenous apoptosis inducers, a fact that may be ascribed to an adaptive response and/or selection of resistant clones. In this section, we will briefly discuss how Env, Vpr, Tat, and PR induce MMP and cell death.

Env

Env and its soluble product gp120 have been thought for long to be the principal HIV-1 encoded killer protein. Three mechanisms have been proposed for the action of Env. First, Env-mediated interaction of virions with the cell surface has been suggested to perturb ion fluxes of the plasma membrane, leading to ballooning of the cell and its necrotic demise. [58] Second, interaction of the gp120/gp41 envelope complex, present on the membrane of HIV-infected cells, with the CD4/CXCR4 complex of uninfected cells, could mediate a cell type-dependent pro-apoptotic signal transduction process. Thus, cross-linking of CD4 or CXCR4 causes a rapid $\Delta\Psi_m$ disruption and apoptosis in lymphocytes. [21] gp120-mediated cross-linking of CD4 causes a decrease in Bcl-2 expression in T cells. [59] Soluble gp120 also triggers calcium mobilization, ATP depletion, decline in $\Delta\Psi_m$, and neurotoxicity in fetal monolayer cultures from a number of brain regions. [60] Third, interaction of the gp120/gp41 complex present on HIV-infected

cells with the CD4/CXCR4 complex of uninfected cells can result in cell-to-cell fusion. [61] Such syncytia then undergo apoptosis after a lag phase which depends on the cell type involved in the fusion process. [54] It remains an open possibility that the nonphysiological formation of syncytia by itself, rather than acute signals mediated via gp120/gp41 in interaction, accounts for cell death. Syncytia arising from the fusion of HeLa cells expressing the HIV-1 encoded Env with cells expressing the CD4/CXCR4 complex spontaneously undergo apoptosis in a process which is accompanied by MMP. [54] Caspase inhibition does not suppress the AIF- and cytochrome *c*-translocation, yet prevents all signs of nuclear apoptosis. Bcl-2-mediated inhibition of MMP also prevents the subsequent nuclear chromatin condensation and DNA fragmentation. This suggests that MMP occurs upstream of caspase activation in syncytial apoptosis. Moreover, we have found that the release of AIF occurs before that of cytochrome *c* and before caspase activation. [54] Microinjection of AIF into syncytia suffices to trigger rapid, caspase-independent cytochrome *c* release. Neutralization of enogenous AIF by injection of an AIF-specific antibody prevents all signs of spontaneous apoptosis occurring in syncytia, including the mitochondrial cytochrome *c* release and nuclear apoptosis. In contrast, cytochrome *c* neutralization prevents only nuclear apoptosis and does not affect the release of AIF. Taken together, our results establish that the following molecular sequence governs apoptosis of Env-induced syncytia: Bax-mediated/Bcl-2-inhibited MMP → AIF release → cytochrome *c* release → caspase activation → nuclear apoptosis. [54]

Vpr

If T cells are infected with vesicular stomatitis virus envelope G (VSV-G) glycoprotein-pseudotyped HIV-1 *in vitro*, Vpr becomes rate-limiting for cell killing. [62-64] The 14 kD Vpr protein (96 amino acids) kills lymphocytes, [63] monocytes, [62] and neurons, [65] either upon infection with *vpr* positive HIV-1 isolates [62-64] or upon extracellular addition of the Vpr protein. [65,66] Virion-associated Vpr may cause apoptosis in the absence of viral replication. [67]

We have discovered recently [68] that synthetic Vpr, when added to intact cells or to purified mitochondria, causes a rapid dissipation of the mitochondrial transmembrane potential ($\Delta\Psi_m$), as well as the mitochondrial release of cytochrome *c* and AIF. This effect is mimicked by the C-terminal moiety of the molecule (Vpr52-96), not by its N-terminal moiety (Vpr1-51), and is inhibited by overexpression of Bcl-2. Vpr52-96 also causes a rapid $\Delta\Psi_m$ loss when added to intact cells, and this effect precedes the induction of apoptotic nuclear chromatin condensation (Fig. 2). If added to lymphocytes or COS cells, a substantial fraction of FITC-labeled Vpr52-96 binds to mitochondria. [68] A biotinylated Vpr-derived peptide (Vpr52-96) may be employed as a bait to specifically purify a mitochondrial molecular complex containing ANT. Surface plasmon resonance indicates that the Vpr C-terminus binds purified ANT with an affinity of 7.4×10^8 M^{-1}. We have found that Vpr favors the permeabilization of proteoliposomes containing ANT. This effect is enhanced by addition of recombinant Bax and prevented by recombinant Bcl-2. In addition, *Saccharomyces cerevisiae* strains lacking ANT are less susceptible to Vpr52-96-induced killing than control cells, yet recover Vpr52-96 sensitivity when retransfected with ANT. Thus, Vpr kills cells via a direct effect on the mitochondrial membranes and in particular on ANT. [68] The same structural motifs relevant for cell killing are responsible for the mitochondriotoxic effects of Vpr. Substitution of critical Arg residues (R73, R80) in the mitochondriotoxic domain (aa 71-82) strongly diminishes both the mitochondrial and the cytotoxic effects of Vpr. [68] These observations

correlate with the facts that R80 mutations reduce cell killing by VSV-G pseudotyped HIV-1 *in vitro* [63] and that R73 and R80 are extremely conserved among pathogenic HIV-1 isolates. Intriguingly, the same domain that we have implied in the apoptogenic activity of Vpr is also critical for the G2 arrest of the cell cycle induced by Vpr. [63] Since G2 arrest and apoptosis induction by Vpr have similar structural requirements, it is tempting to speculate that both Vpr effects (cytotoxicity and cell cycle arrest) may be functionally interconnected at the level of mitochondria.

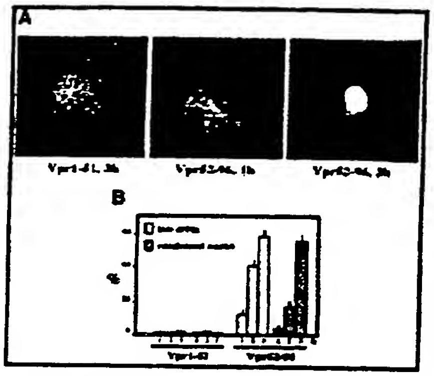

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FIGURE 2. Induction of mitochondrial and nuclear signs of apoptosis by Vpr. COS cells were cultured in the presence of 1 µM Vpr52-96 (or as a negative control Vpr1-51) for 1 or 3 h and stained during the last 30 min of culture with Hoechst 33324 (which detects nuclear chromatin condensation: blue fluorescence) and the $\Delta\Psi_m$ sensitive dye JC-1 (which marks mitochondria with a high $\Delta\Psi_m$ in red, with Vpr1-51 at 3 h, and mitochondria with a low $\Delta\Psi_m$ in green, with Vpr52-96 at 1 h). Note that the dissipation of the $\Delta\Psi_m$ is detectable well before nuclear apoptosis becomes visible (at 3 h with Vpr52-96). Representative images are shown in A, and the kinetics of the mitochondrial and nuclear signs of apoptosis ($X \pm SEM$, $n = 3$) are shown in B.

Tat

Cells stably transfected with Tat are more susceptible than control cells to undergo apoptosis upon serum withdrawal. According to one study, Tat causes a decrease in Bcl-2 expression and an increase in Bax expression. [69] Another study reports that Tat translocates to mitochondria concomitantly with the $\Delta\Psi^m$ loss. [70] Addition of extracellular Tat causes apoptosis, at least in some cell types. [71] Tat down-regulates the mitochondrial isoform of superoxide dismutase (SOD2), either at the post-transcriptional [72] or transcriptional levels. [73] Down-regulation of SOD2 is thought to sensitize cells to the lethal effects of reactive oxygen species produced in mitochondria. Exogenous Tat can also induce the expression of pro-apoptotic proteins. Such Tat-induced proteins include CD95 (in T cells) [74,75] and Par-4 (in cultured hippocampal neurons), which induces apoptosis via a mitochondrion-dependent mechanism. [76,77] These results suggest multiple connections between Tat-induced cell death and MMP.

PR

Transfection-enforced overexpression of HIV-1-encoded protease (PR), a cysteine protease essential for viral replication, can induce cell death. [78] According to one report, cell death is preceded by PR-mediated cleavage of the anti-apoptotic, MMP-inhibitory protein Bcl-2 between Phe112 and Ala113. [79] Clipping of the BH4 domain may be expected to neutralize Bcl-2 or to transform it into a pro-apoptotic, MMP-inducing protein, as this has been demonstrated for a cleavage event caused by caspase-3. [80,81]

Based on the results summarized above, it appears that HIV-1 uses several independent strategies to induce MMP and apoptosis (Fig. 3). However, it remains an ongoing conundrum whether these manifold strategies are designed to cooperate among each other in an additive or synergistic fashion, in the same cell.

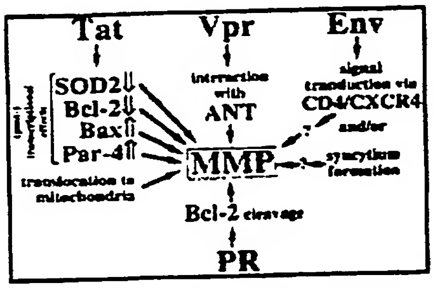

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FIGURE 3. Indirect and direct effects of HIV-1 encoded proteins on mitochondria. Tat, Vpr, Env, and PR may induce mitochondrial membrane permeabilization (MMP) via a variety of different mechanisms. For details and references consult main text.

VIRAL PROTEINS ACTING ON MITOCHONDRIA: A GENERAL STRATEGY OF APOPTOSIS MODULATION?

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Why, in teleological terms, do viruses regulate apoptosis? It appears obvious that, especially at the beginning of the viral life cycle, before lysis, the virus must aim at preventing its host cells from dying. However, the virus may trigger apoptosis at late stages of the viral life cycle, either to facilitate viral spreading to adjacent phagocytic cells (which recognize and engulf the dying cell) or to hijack the phagocytic system (which may be overwhelmed by apoptotic bodies). In the particular case that the infected host cell belongs to the non-specific defense system (e.g., phagocytes) or to the immune system, the virus may employ a strategy to induce rapid cell death shortly after infection. In other words, the virus could kill antiviral cells and productively infect (or persist in) other cells, not involved in antiviral defense. Here, we will briefly discuss the current literature on mitochondrial effects mediated by viruses other than HIV-1.

Viral Apoptosis Inhibitors Acting on Mitochondria

Recent studies have unravelled the existence of several viral apoptosis inhibitors acting on mitochondria. Several pathogenic viruses produce apoptosis inhibitory (and presumably MMP-inhibitory) Bcl-2 analogues (reviewed in ref. 34): adenovirus (protein: E1B19K), african swine fever virus (5-HL/A179L), herpesvirus saimiri (HVS-Bcl-2), Kaposi sarcoma-associated herpes virus 8 (Ksbcl-2), and murine gammaherpesvirus-68 (M11). Epstein-Barr virus even encodes two structural and function homologues of Bcl-2: BHRF1 and BALF1. Among these proteins, HVS-Bcl-2 and 5-HL/A179L have been described

to inhibit MMP, much as Bcl-2 does. According to our working hypothesis, Bcl-2 prevents MMP via its interaction with the adenine nucleotide translocator (ANT). [82] It is tempting to speculate that viral Bcl-2 analogues inhibit MMP via a similar mechanism. A cytomegalovirus inhibitor of apoptosis (CIA), without any obvious sequence homology to Bcl-2, has been found to bind to ANT, [83] suggesting that it acts as a functional Bcl-2 homologue. Another mechanism of apoptosis inhibition may be the neutralization of pro-apoptotic proteins from the Bcl-2 family. As an example, the large T antigen from simian virus 40 (SV40) binds a pro-apoptotic member of the Bcl-2 family, p193. [84]

Viral Apoptosis Inducers Acting on Mitochondria

An influenza virus B protein (PB2) has been the first viral protein to be shown to interact with mitochondria via its N-terminus. [85] This is an interesting finding because influenza virus B is one of major causative agents of Reye's syndrome, an acute mitochondriopathy associated with massive cell death. [19] However, the exact effects of this influenza virus protein have not been determined in molecular terms. More recently, it has been found that the p13 (II) protein derived from the X-II ORF of HTLV-1 is targeted to mitochondria, where it induces a dissipation of the $\Delta\Psi_m$ and matrix swelling. [86] Mitochondrial targeting of this protein has been mapped to a decapeptide sequence which contains several Arg residues that are asymmetrically distributed in an α-helix and which resemble the mitochondriotoxic motif of HIV-1-encoded Vpr (Fig. 4). In contrast with Vpr, however, Arg$\rightarrow$Ala substitutions did not abolish the mitochondrial targeting of p13. [86] Hepatitis B virus X protein (HBV-X) is a potent apoptosis inducer whose effect is neutralized by Bcl-2 overexpression. [87] HBV-X protein colocalizes to mitochondria with a human voltage-dependent anion channel (VDAC) isoform, HVDAC3, and alters its transmembrane potential. [88] Thus, HBV-X may act in a similar fashion as it has been suggested for Bax, a Bcl-2 antagonist that may cause VDAC to form a large protein-permeant channel. [89] Intriguingly VDAC can interact with ANT, [90] suggesting that HBV-X targets the same molecular complex as Vpr, CIA, and viral Bcl-2 analogues.

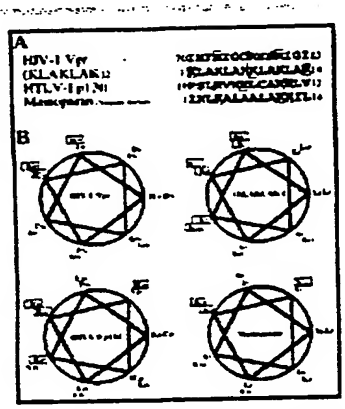

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FIGURE 4. Comparison of the mitochondriotoxic domains of four proteins or peptides known to induce MMP. The primary structure is shown in A and the helical wheel presentation in B. Positively charged residues are boxed. Numbers refer to the position of each amino acid in the entire protein or peptide. Note the common amphipathic distribution of residues in each of the α-helices.

The above examples clearly illustrate that viruses may target different mitochondrial proteins to induce or prevent apoptotic MMP. Clearly, our current information on the submolecular mode of action of these apoptosis regulators is scarce. Future investigation will identify additional virus-encoded MMP regulators and determine to which extent mitochondrial targeting of viral proteins impinges on the viral life cycle. Irrespective of these general considerations, it becomes clear, however, that mitochondria may constitute a specific target of HIV-1 mediated cytopathogenicity.

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FOOTNOTES

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